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# *Trichomonas vaginalis* origins, molecular pathobiology and clinical considerations

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**Abstract:**

*Purpose of review:* We aim to integrate a selection of the most recent data on *Trichomonas vaginalis* (TV) origins, molecular cell biology and TV interactions with the urogenital tract microbiota with trichomoniasis symptoms and clinical management.

*Recent findings:* Transcriptomics and proteomics datasets are accumulating facilitating the identification and prioritisation of key target genes to study TV pathobiology. Proteins involved in host sensing and cytoskeletal plasticity during TV amoeboid transformation were identified. TV was shown to secrete exosomes and a macrophage migration inhibitory factor-like protein that both influence host-parasite interactions. TV co-infections with *Mycoplasma* species and viruses were shown to modulate the inflammatory responses while TV interactions with various *Lactobacillus* species inhibit parasite interactions with human cells. TV infections were also shown to be associated with bacterial vaginosis (BV). A broader range of health sequelae is also becoming apparent. Diagnostics for both women and men based on molecular approaches are being refined, in particular for men.

*Summary:* New developments in the molecular and cellular basis of TV pathobiology combined with data on the urogenital tract microbiota and immunology have enriched our knowledge in human-microbe interactions that will contribute to increasing our capacity to prevent and treat TV and other STIs.

**Keywords:**

Omics data, *Mycoplasma*, *Lactobacillus*, *microbiota*, Trichomonasvirus.

**Abbreviations:**

Extracellular matrix (ECM); lipophosphoglycan (LPG); MIF macrophage migration inhibitory factor; nucleic acid amplification tests (NAATs); sexually transmitted infections (STI); tetraspanin (TSP); *Trichomonas vaginalis* (TV); *Trichomonas tenax* (TT); *Trichomonas vaginalis* virus (TVV); vaginal epithelial cell (VEC).

**Introduction**

*Trichomonas vaginalis* (TV) is the most common non-viral sexually transmitted infection (STI) worldwide. The World Health Organisation estimated that in 2005 there were 248 million cases of TV and this had increased by 11.2% to 276.4 million cases in 2008 [1]. There is significant regional variation with the highest rates in the Americas with an incidence of 177.7 per 1000 females and 180.6 per 1000 males aged 15 to 44 and a prevalence of 22% in females and 2.2% in males. This contrasts with South East Asia where the incidence is estimated at 40.3 per 1000 females and 50.1 per 1000 males with a prevalence of 5.6% and 0.6% respectively. Notably TV is affecting primarily woman of disadvantaged populations in both affluent and resource limited countries and is still very much a neglected disease despite increasing awareness of its important health sequelae.

The availability, for one TV strain, of a draft genome sequence and annotation [2] have stimulated numerous studies on the parasite population genetics and molecular basis of it pathobiology [3, 4]. These developments paralleled with studies on the urogenital microbiota and immunology will eventually converge to improve our knowledge of TV pathobiology and assist with prevention and treatment protocols. Here we present an initial integration of a selection of the most recent developments in basic research on the parasite molecular cell biology with its clinical symptoms and management.

**TV as a bird derived zoonosis?**

A number of different molecular phylogenies have surprisingly supported a close relationship between TV and parasites isolated from birds [5]. The two human parasites TV and *Trichomonas tenax* (TT, from the oral cavity) were thought for some time to be closely related to, and possibly derived from, each other [2, 5]. Molecular phylogenies established that TV and TT are more closely related to distinct species of *Trichomonas* isolated from birds then they are to each other (Figure 1). This suggests two independent zoonotic origins from birds for these two human parasites. Hence it will be of great interest to perform comparative studies of the genomes and host-parasite interactions between human and bird *Trichomonas* species to identify the molecular basis

of their respective pathobiologies. Thus TV is likely join the long list of human pathogens derived from birds [6]. These considerations further illustrate the importance of studying pathogens in an integrated way across the fields of medical and veterinary research [6].

**Genomics and allied omics**

The draft genome sequence data and annotation for one clinical TV strain uncovered a surprisingly large and highly repetitive genome for a microbial eukaryote (Table 1) [2, 4]. These data represent an invaluable source of information to study the epidemiology and molecular cell biology of host-TV interactions [3]. Only a selection of recent developments is discussed here and complementary reviews cover a broader range of issues [3, 4, 7-9]. Due to TV vast potential protein coding capacity (Table 1), transcriptomics and proteomics investigations are particularly pertinent to identify and prioritise the functional characterisation of key TV proteins enabling infections [3, 4].

Two recent studies have initiated the global profiling of TV gene transcription in various *in vitro* growth conditions for two distinct TV strains using RNA-seq methodologies [10, 11]. Circa 30,000 genes were transcribed in the various tested conditions in each strain [10, 11] and a core set of about 20,000 genes was identified across the 11 conditions considered in one study [10]. Modulation of mRNA levels upon exposure to oxidative stress, binding to vaginal epithelial cells (VEC) or glucose restriction have identified hundreds of genes involved in responses to these environmental cues. These included genes mediating oxidant scavenging and cytoskeletal re-arrangements upon exposure to oxygen, binding to VEC or a combination of both [10], or amino acid metabolism upon glucose restriction [11]. Notably the combined exposure of TV to oxygen and VEC stimulated the broadest set of TvBspA genes up-regulation [10], consistent with the hypothesis that the largest gene family (911 genes) encoding candidate surface proteins are important in host-TV interactions [12]. A selection of TvBspA genes are listed in Supplementary digital content 1 (SDC1), illustrating the striking variation of transcription for some genes between the two studied strains, possibly underlying antigenic variation [7, 12]. The modulation of the actin-based cytoskeleton upon contact to VEC at the transcription level was also highlighted [10]. TV trophozoites

binding to VEC rapidly differentiates into an amoeboid form maximising contact with host cells, a key step in the infection process (Figure 2). The fimbrin protein (an actin bundling protein) was recently shown to dramatically change its cellular distribution from the cell periphery in trophozoites to focal points co-localising with actin in the amoeboid form [13].

With a highly repetitive genome possessing numerous transposable elements (TE), it is important to highlight here the recent analyses of one family of TE (class II Tc1/mariner) and their impact on gene transcription across 94 TV isolates [14]. Several insertions of TE were demonstrated to reduce or abolish the transcription of neighbouring genes, suggesting that such TE driven mutations could have functional implications and contribute to adaptive evolution of the parasite [14]. Combining comparative genomics and transcriptomics across a number of strains will be required to assess the role of TE in mediating TV genetic and phenotypic diversity.

An annotated genome also facilitates proteomics investigations and a number of such datasets have been published [3, 7, 9]. Here we consider the most recent data on surface proteins and cargo proteins of the secreted exosomes.

**Surface and secreted proteins and exosomes**

Surface and secreted proteins from the parasite are at the forefront of host-parasite interactions; hence these proteins have attracted great interests [7-9]. Two recent insights into the molecular basis of host-parasite interactions are considered here.

Gene annotations identified a total of 11 TV tetraspanin (TSP) proteins possessing the characteristic four transmembrane domain of TSPs and two strains transcribe slightly different set of these genes (SDC1). Based on the numerous role of TSP proteins in human and model systems in orchestrating cell adhesion, fusion, signalling, proliferation and migration and their status as markers of exosomes, identified TvTSP as important target proteins to study host-TV interactions [15, 16]. Cell surface proteomics confirmed three TvTSP proteins [15] and one TvTSP protein, TvTSP6, was functional investigated [16]. The TvTSP6 cellular re-distribution (from flagella to the main cellular body and large intracellular vesicles) upon TV binding to VEC and its role in regulating TV migration, makes it a prime target to further investigate how TV

sense and orchestrate its binding to, and migration through, human tissues during infection [16]. The TvTSP6 and the fimbrin re-localisation data [13] illustrate the dramatic cellular changes taking place in TV upon binding to host cells.

The secretion of exosomes by TV was investigated through a combination of cellular and proteomics approaches and identified exosomes with cargo RNA and a total of 215 cargo proteins including TvTSP1 and TvBspA_TVAG_216280 [17], the most and second most highly transcribed genes of these two gene families, respectively, in the recent RNA-Seq data (SDC1). Remarkably, TV exosomes were shown to bind to host cells, to modulate the TV-TV binding and TV-VEC and TV-prostate cells binding properties and to have immunomodulatory properties making these microvesicles and their cargo proteins attractive targets to gain new insights into TV pathobiology [17]. These various data identify the two exosomal membrane proteins TvTSP1 and TvBspA_TVAG_216280 as potential antigens for vaccine developments [18].

Another secreted factor likely to be important in modulating host-TV interactions is the TV macrophage migration inhibitory factor (TvMIF_ TVAG_219770) that was recently shown to be similar to human MIF and functionally investigated [19]. Notably the TvMIF gene is highly transcribed whereas a related pseudogene is not (SDC1). Functional characterisation of the TvMIF protein demonstrated that it was active in inhibiting macrophage migration, stimulating host cell signalling, was interacting with the human CD47 MIF receptor and increased the growth and migration through matrigel of both benign and prostate cancer cells [19]. The data also demonstrated an adaptive immune response to the TvMIF in TV positive patients, consistent with the protein being secreted during infections. Could different TV strains express variant TvMIF genes in response to this host response? If so this might rationalize the TvMIF pseudogene (SDC1). Taken together these data are consistent with TvMIF stimulating inflammation and host cell proliferation and could be responsible for the underlying TV association with aggressive prostate cancers [19].

**TV-microbes interactions and their impact on host-TV relations**

It is increasingly recognised that studying host-parasite-bacteria-virus complex interactions at mucosal surfaces represent an important new paradigm to comprehend the impact, positive and negative, of these interactions on human health and disease conditions, and TV is no exception [20](Figure 2). Three recent developments are of interest in this context. First TV interactions with *Mycoplasma hominis* have been shown *in vitro* to synergistically up-regulate macrophage pro-inflammatory responses [21]. The pro-inflammatory responses to the TV-*Mycoplasma* consortium on immune, and possibly epithelial, cells might be clinically relevant by contributing to various health sequelae associated with TV infections (see later sections). Notably a recent metagenomics investigations of the vaginal microbota led to the characterisation of the genome sequence of a new species of *Mycoplasma* that is strongly associated with TV infections [22]. This suggests that different TV-*Mycoplasma* consortia might exist. Could these lead to variations in inflammatory responses [21] and variations in TV gene transcription and protein synthesis profiles?

Second, endogenous TV viruses (TVV) isolated as TV-TVV consortium from symptomatic female patients were also shown *in vitro* to stimulate pro-inflammatory responses in human VEC [23]. Notably metronidazole treatments amplified the in-vitro pro-inflammatory responses in TVV positive parasites possibly due to the increase in TVV particles released from dying parasites. This phenomena could explain the negative impact of drug treatments observed during pregnancy [23](see later sections).

Finally, the effect of different Lactobacilli in *in vitro* TV-VEC interactions assay was investigated and demonstrated that the bacteria mostly associated with healthy vaginal microbiota inhibited these interactions, consistent with protective functions of Lactobacilli species, with the exception of *Lactobacillus pentosus*, which enhanced TV adhesion to VEC [24]. These observations were shown to be Lactobacilli dose dependent [24]. Consistent with these data TV infections were shown to be associated with bacterial vaginosis, a dysbiosis characterised by the loss of protective Lactobacilli [25, 26].

These different considerations suggest that diagnostics will need in the future to integrate TV, bacteria (mutualists and pathogens) and viruses (from both TV and humans) involved in the complex microbial ecology of the human

urogenital tracts, possibly by adapting metagenomics approaches currently exploited to characterise the complex host-microbiota-pathogens interactions e.g. [22]. So for example differentiating TV infections from TV-TVV, TV-Mycoplasma or TV-TVV-Mycoplasma co-infections could benefit patients by pointing to differential treatments minimising inflammatory sequelae in those different contexts [23].

**Impact on human health**

Previously regarded as a nuisance for women, it is now recognised that TV has a number of presentations in both genders, is a risk factor for HIV transmission and acquisition, as well as being associated with adverse pregnancy outcomes. TV infects the squamous epithelium of the genital tract, often including the female urethra. The usual symptoms of TV are vaginal discharge in women associated with vaginitis, cervicitis, and urethritis, although it is recognised that up to 1/3 women have no symptoms [27]. Recent studies have suggested an increased risk of acute endometritis in association with TV [28]. In men, TV is usually a transient infection and studies suggest up to 75% of men are asymptomatic but it may be associated with urethritis, rarely with balanitis [29] and possibly prostatitis [30]. A number of case reports suggest a widening of the pathologic spectrum of TV in humans including vulval ulceration [31], adult conjunctivitis [32] and pulmonary infections in association with HIV [33] and there is discussion around the possible contribution of TV to aggressive prostatic cancers [19, 34].

TV in pregnancy has been associated with low birth weight, premature rupture of membranes and preterm delivery [35, 36]. However some studies have failed to show that treatment of TV improves pregnancy outcome and some have indicated that treatment of TV infection in pregnancy may have a negative impact on the pregnancy [37, 38] - possibly explained by TVV inducing strong pro-inflammatory response upon drug treatment discussed earlier [23]. Neonatal infection, from vertical transmission with TV is rare. It usually causes a urogenital infection and there are case reports of vulvitis and

positive urine cultures in neonates. There are occasional reports of TV causing neonates respiratory tract infection [39].

Multiple reports support an epidemiological association between HIV and TV. There is growing evidence that TV infection may enhance HIV transmission and acquisition and there may be an increased risk of TV infection in those that are HIV infected [40-42]. In women with vaginal TV who are HIV positive there is some evidence that TV may be more difficult to treat. A randomized clinical trial found that a 2g single oral dose of metronidazole was not as effective as 500mg of metronidazole twice daily for 7 days for trichomoniasis among HIV-infected women [43].

**Diagnostics**

The choice of specimen, diagnostic test and testing strategy for detection of TV will depend on the patient population, clinical setting, and the available laboratory facilities. In settings where there is a high prevalence of STIs nucleic acid amplification tests (NAATs) are now the test of choice where resources allow and have become the 'gold standard' offering the highest sensitivity for the detection of TV [44-47].  In settings where molecular testing is not available the diagnosis of TV infection remains problematic because wet mount microscopy alone performs poorly for the diagnosis of trichomoniasis in women. Wet mount microscopy and current rapid antigen detection tests are not suitable for diagnosis of trichomoniasis in men.

A number of commercially available NAAT assays have sensitivities of 95–100% and the specificity is also 95–100% depending on the specimen and reference standard.  The APTIMA TV Assay (Hologic Gen-Probe, San Diego, CA) was FDA-cleared in 2011 for use with urine, endocervical and vaginal swabs, and endocervical specimens collected in the Hologic PreserveCyt solution (ThinPrep) from females only. The BD ProbeTec Trichomonas Vaginalis Qx Amplified DNA Assay (Becton Dickinson, Franklin Lakes, NJ) launched in Europe (EU cleared) in 2012. The diagnosis of TV in men has been challenging given the low sensitivity of microscopy and lack of FDA clearance to date for any NAATs or point-of-care tests for use with male

specimens. Some laboratories have verified the performance characteristics of NAATs through a validation process for male urine specimens or penile-meatal swabs [45].

Culture, until recently, has been considered 'the gold standard,' with specificity approaching 100% but the sensitivity can be as low as 75% compared with molecular testing. Culture systems such as InPouch TV (BioMed Diagnostics, San Jose, CA) allow for direct inoculation, culture and microscopic examination. Such systems are useful when immediate transportation of specimens to the laboratory is not available. The specimen should be inoculated within an hour of collection to maintain viability of the parasite [46].

A number of point of care tests that detect TV antigens or nucleic acids have been recently developed that allow for extended time between specimen collection and testing and more flexible sample storage temperatures. Commercially available tests include the OSOM Trichomonas Rapid Test (Sekisui Diagnostics, California, USA) and the TV latex agglutination test (Kalon Biological, Surrey, UK) and the Affirm VPIII (Becton Dickinson, Maryland, USA).  The sensitivities of these tests are similar to culture and consistently higher than wet mount microscopy. The specificities range from 92% to 100%. All three tests are intended for use with vaginal swabs from symptomatic women. However, none has been evaluated for screening asymptomatic women or for the diagnosis of TV in men.

Traditionally the diagnosis of TV has been made by the detection of the motile parasites on a  wet mount preparation of vaginal or urethral secretions by light-field microscopy. Microscopy has the advantage that it is cheap, and can be performed near to the patient in a clinic setting.  The specificity with trained personnel is high. However specimens should be examined within 10 minutes and the sensitivity is reported to be as low as 45-60% in women and even lower in men.

**Treatment**

Treatment for TV is with nitroimidazoles. As TV in women frequently infects the urethra and paraurethral glands and cure rates of around 50% with metronidazole intravaginal gel are reported, systemic treatment is required. A Cochrane review found a parasitological cure in >90% of cases of TV with almost any nitroimidazole drug given as a single oral dose or over a longer period [48]. Single dose treatments are associated with more frequent side effects than longer oral treatment. Standard treatments are a 5 to 7 day course of metronidazole 400 to 500 mg twice daily or 2g of metronidazole or tinidazole as single dose [49, 50].

Meta-analyses have concluded that there is no evidence of teratogenicity from the use of metronidazole in women during the first trimester of pregnancy [51]. Metronidazole can be used in all stage of pregnancy and during breastfeeding. Metronidazole enters breast milk and may affect its taste. The manufacturers recommend avoiding high doses if breastfeeding or, if using a single dose of metronidazole, breastfeeding should be discontinued for 12-24 hours to reduce infant exposure. Tinidazole is pregnancy category C (animal studies have demonstrated an adverse event) and its safety in pregnant women has not been well-evaluated.

Persistent or recurrent TV may be due to inadequate therapy, re-infection, or resistance [52]. Development of resistance against nitroimidazoles can be due to aerobic and anaerobic resistance. In the USA, it is estimated that 5% of clinical isolates of TV exhibit some degree of metronidazole resistance, predominantly low level [53]. In vitro resistance does not predict clinical response to treatment, as it may be relative rather than absolute, and may be overcome by high dose metronidazole or tinidazole therapy. Clinical isolates resistant to metronidazole can be resistant to tinidazole but usually with significantly lower minimal lethal concentrations to tinidazole than metronidazole [54, 55].

**Conclusion**

Molecular cell investigations, greatly facilitated by the availability of the genome sequence, have brought important new insights into TV pathobiology.

These developments combined with investigations on the microbiota and immunology of the urogenital tract have started to draw a more precise picture of the complex interplay between pathogens, the microbiota and the immune system. It is imperative to integrate these different data to improve our knowledge on human-microbe interactions to eventually improve our capacity to avoid and treat STIs that affect hundreds of millions of people of all ages, in particular those living in resource limited conditions.

**Key points:**

- Genomics, transcriptomics and proteomics datasets have started to boost research efforts characterising the molecular and cellular basis of TV pathobiology
- Complex interplay between TV, bacteria and viruses are being uncovered
- Future research efforts in the laboratory and the clinic will need to consider the complex interplay between TV, bacteria and viruses
- Diagnostic and clinical management of TV will benefit from basic research
- Improved diagnostic tests will enhance our understanding of the disease spectrum caused by TV

**Conflict of interest**

There are no conflicts of interest.

**Figure legends**

**Figure 1. Molecular phylogeny identify bird parasites as sister taxa to TV.**

Molecular phylogeny based on an Rpb1 (the largest subunit of the RNA-polymerase II) protein alignment focusing on Trichomonadea are consistent with phylogenies derived from other genes - see Maritz et al. [5] for details including accession number of sequences. Species isolated from humans are highlighted with arrows; all other species were isolated from birds (bird species and country are indicated). Note one sequence derived from a bird isolate that is highly similar to TV sequences, which could represent a transfer from human to bird.

**Figure 2. Host-TV interactions and its interplay with other microbes**
A. Upon contact with human tissues (epithelial cells, extracellular matrix proteins [ECM]) TV trophozoites (pear-shaped cells) rapidly differentiate into amoeboid forms (pancake-like cells), dramatically increasing surface contact. This is one of the cellular processes thought to be essential for long-term infections [13, 20]. A combination of TV lipophosophglycans (LPG) and surface proteins mediate the binding to host tissues. Some TV proteins are delivered to the host cell surface via TV exosomes and thought to influence TV binding (see main text for these different points). B. Complex interplay between Bacteria, Viruses and Eukaryote in human-microbes interactions. The diagram illustrates a TV-infected pregnant woman and her partner in the global context of human-microbes interactions in their respective urogenital tracts. TV infections can affect the health status of both adults and the embryo (eg preterm birth and HIV transmission in utero). Other common eukaryotes encountered in the urogenital tract are Fungi of the genus *Candida*. TV, TVV, *Mycoplasma*, and other microbes can modulate the inflammatory status of the urogenital tracts and by doing can alter the susceptibility of a given individual to additional infectious agents such as HIV – see main text. Modified figure derived from [20].

**Table 1.** Some key features of the TV genome

**Supplementary Digital content 1 (SDC1).** Comparison of transcript abundance between two different TV strains for selected genes.

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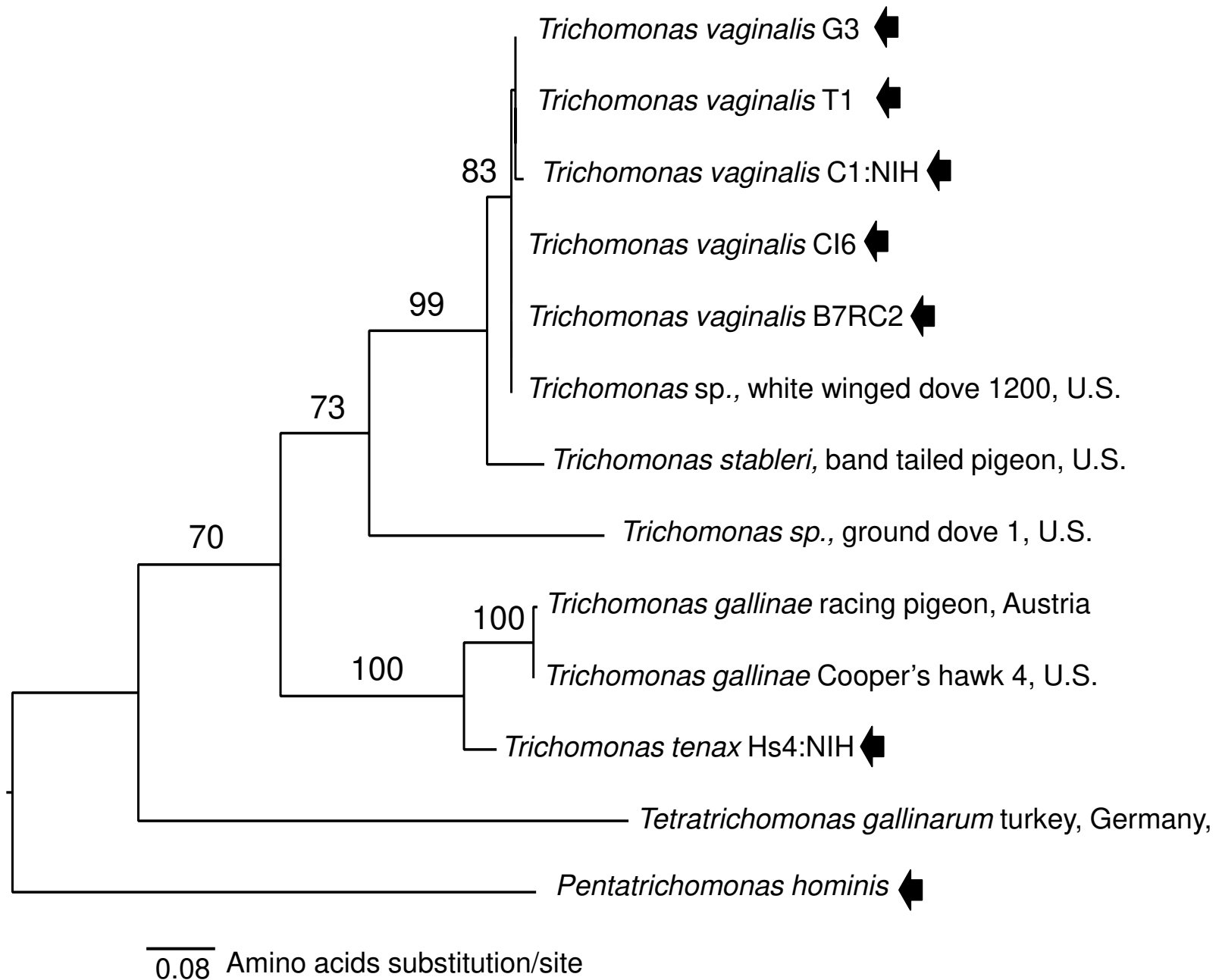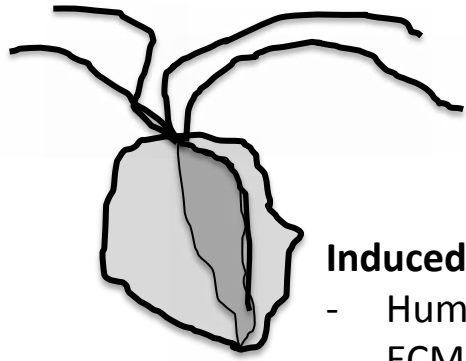
Figure 1



*Trichomonas vaginalis* G3

*Trichomonas vaginalis* T1

83 *Trichomonas vaginalis* C1:NIH

*Trichomonas vaginalis* CI6

99 *Trichomonas vaginalis* B7RC2

73 *Trichomonas* sp., white winged dove 1200, U.S.

*Trichomonas stableri,* band tailed pigeon, U.S.

70 *Trichomonas sp.,* ground dove 1, U.S.

100 *Trichomonas gallinae* racing pigeon, Austria

100 *Trichomonas gallinae* Cooper's hawk 4, U.S.

*Trichomonas tenax* Hs4:NIH

*Tetratrichomonas gallinarum* turkey, Germany,

*Pentatrichomonas hominis*

0.08 Amino acids substitution/site

Figure 2
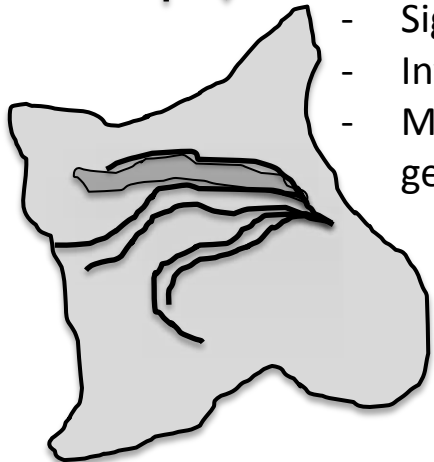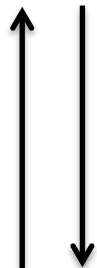
**A**

Trophozoite

**Induced by binding to:**
- Human cells
- ECM proteins

**TV-tissue binding modulated by:**
- LPG and surface proteins
- Exosomes

**Amoeba transformation:**
- Signalling with tetraspanin
- Involves fimbrin
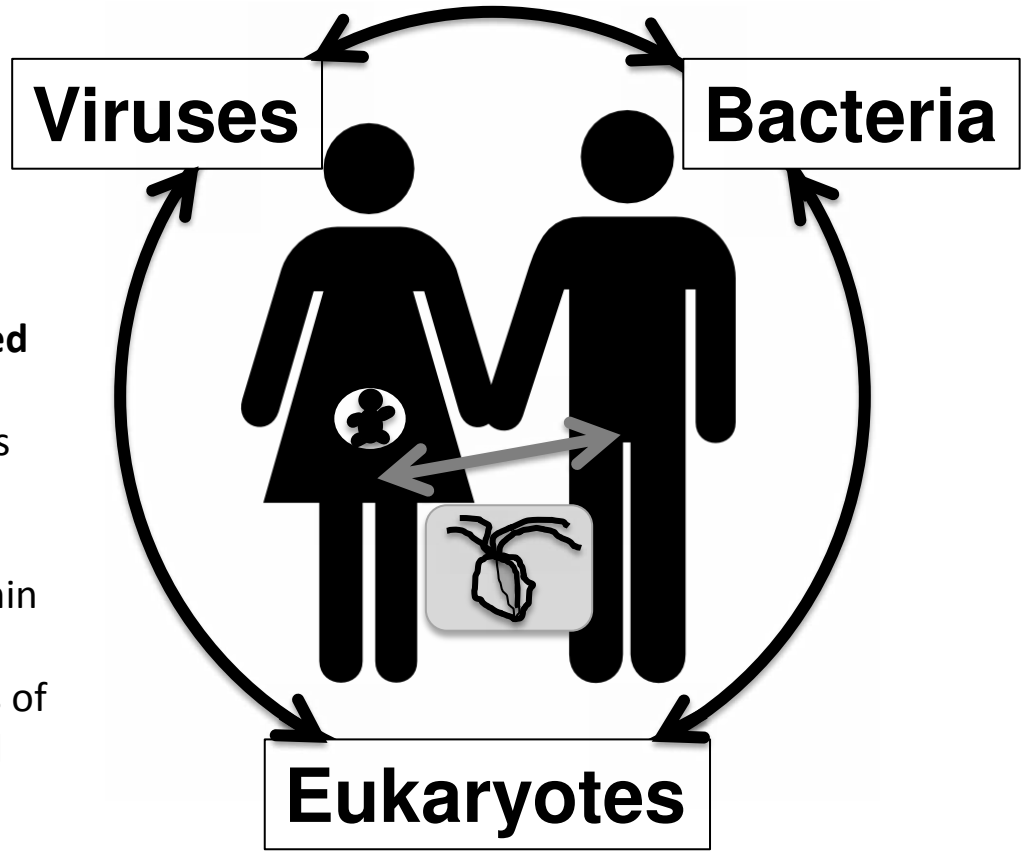- Modulation of hundreds of genes at the mRNA level

Amoeba

**B**

| TVV dependent inflammation Boosting of HIV transmission | TV induced dysbiosis *Mycoplasma* transmission |

Viruses

Bacteria

Eukaryotes

TV dependent inflammation & tissue damage
Synergism with *Mycoplasma*

Table 1

**Table 1. *Trichomonas vaginalis* genome features compared with selected species**

| Species | Predicted gene number (ORF) | Genome size (Mbp) | % of genome that is repetitive |
|---|---|---|---|
| *Trichomonas vaginalis** | 59681 | 160 | 65 |
| *Homo sapiens* | 35845 | 2900 | 46 |
| *Drosophila melanogaster* (fruit flie) | 13679 | 180 | 2 |
| *Entamoeba histolytica** | 9938 | 25 | 6 |
| *Giardia lamblia** | 9649 | 12 | ? |

Subset of a table from reference [2]
*Microbial eukaryotes infecting mucosal surfaces